bio-protocol Preprint

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Stereotactic injections of AAV in hippocampal CA1

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An abbreviated version of this protocol was published in Nature Communications in Apr 2021

Neurexins regulate presynaptic GABAB-receptors at central synapses

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Detailed protocol

Materials and Reagents

1. Test mice, aged 21 postnatal days
2. Sterile Alcohol Prep Pad
3. Anesthetic (Avertin 125-300 mg/kg; Ketamine hydrochloride 80-100 mg/kg, or Isoflurane1-4%, according to animal protocol)
4. Ophthalmic ointment
5. Vetbond (3M)
6. Local analgesics (Lidocaine)
7. Systemic analgesics (e.g., Buprenorphine SR 0.5-1 mg/kg)
8. Surgical tools

Equipment

1. Stereotactic frame with digital display console and microscope
2. Hair clippers
3. Heating pad
4. Drill
5. Drill bits
6. Harvard Apparatus injection pump
7. Hamilton syringe with tubing filled with mineral oil or Micro injector
8. Pipette puller and pipettes

Procedure

1. Pull a pipette and fill it with mineral oil
2. Secure the pipette on the stereotactic frame and connect it to the Hamilton syringe tubing, avoiding the formation of bubbles in the line.
3. Anesthetize mice with Avertin (125-300 mg/kg) by intraperitoneal injection (or other anesthetics according to approved animal protocol).
4. Inject mouse subcutaneously with analgesics (*e.g.*, Buprenorphine SR)
5. Shave the head of the mouse using standard clippers, sterilizing the scalp with betadine.
6. Position the mouse in the stereotactic frame. Ensure that the head is leveled and straight. Tighten ear bars to secure mouse, being careful to avoid changing the orientation of the head.
7. Apply ophthalmic ointment to the eyes.
8. Apply local anesthesia (Lidocaine) on the incision site.
9. Using sterile micro-scissors, make an incision in the scalp that is long enough to see both bregma and lambda.
10. Zero medio-lateral and anterio-posterior positions of the drill (attached to one arm of the stereotactic frame) at bregma.
11. Drill holes in the skull above the injection site, being careful not to puncture brain. To target the dorsal CA3 we performed 2 injections sites per hemisphere, using the following coordinates from the Bregma: AP: −2.1/−2.1 mm, ML: ±2.0/±2.8 mm, DV: −2.2/−2.1. Injections sites were verified by pilot studies and may vary between experimenters. We recommend performing pilot injections to test the coordinates.
12. Zero needle at bregma, move needle to injection site medio-lateral and anterio-posterior coordinates. Lower needle into brain. Zero dorso-ventral position at the dura.
13. Slowly lower the needle to DV coordinate and start injection (flow rate = 0.35 µl/min; injected volume = 0.8 µl). Wait 2 min after injection completion.
14. Slowly retract the needle.
15. Repeat the injection procedure for the other site and hemisphere.
16. After all injections are completed, drop approximately 1 ml of sterile saline on the mouse skull. Dry with sterile tissue paper.
17. Apply vetbond to close the wound, avoiding the eyes, nose, whiskers, and ears.
18. Place the mouse in a recovery cage and maintain animal temperature at normal with a heating pad. Allow mice to recover from anesthesia before returning them to a holding cage.
19. Check mice daily for signs of infection. Other signs of distress, neurological signs (i.e., ataxia or seizure), or unspecific clinical signs (i.e., as lethargy, low activity, body weight loss, rough coat) will be monitored Follow approved IACUC protocols for humane endpoints, if necessary.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Luo, F. and Sclip, A. (2023). Stereotactic injections of AAV in hippocampal CA1. Bio-protocol Preprint. bio-protocol.org/prep2106.
2. Luo, F., Sclip, A., Merrill, S. and Südhof, T. C.(2021). Neurexins regulate presynaptic GABAB-receptors at central synapses. Nature Communications 0(0). DOI: 10.1038/s41467-021-22753-5